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Commentary

Mitochondrial Biogenesis Drives Tumor Cell Proliferation

Ubaldo E. Martinez-Outschoorn,[*†‡] Stephanos Pavlides,[*†§] Federica Sotgia,[*†] and Michael P. Lisanti[*†‡§]

From the Jefferson Stem Cell Biology and Regenerative Medicine Center,[*] Philadelphia, Pennsylvania; the Departments of Stem Cell Biology and Regenerative Medicine and Cancer Biology,[†] and the Department of Medical Oncology,[‡] Kimmel Cancer Center, Thomas Jefferson University, Philadelphia, Pennsylvania; and the Postgraduate Research Institute of Science, Technology, Environment and Medicine,[§] Limassol, Cyprus

Little is known about the mechanism(s) of arsenic-induced carcinogenesis. A study published by Lee et al[1] in this issue of *The American Journal of Pathology* directly addressed this through study of arsenic-induced Bowen's disease and demonstration of increased mitochondrial biogenesis as a crucial determinant of carcinogenesis. In this study, keratinocyte exposure to low-dose arsenic leads to mitochondrial biogenesis and increased proliferation. Pharmacological or genetic down-regulation of mitochondrial biogenesis abrogates this growth advantage. We discuss these findings in the context of mitochondrial biogenesis in cancer progression and the biological effects of arsenic. Traditionally, cancer cells have been viewed as having impaired mitochondrial function and as relying metabolically on aerobic glycolysis, termed the Warburg Effect. However, there are numerous cancer subtypes with increased mitochondrial oxidative phosphorylation in which enhanced mitochondrial activity is linked to aggressiveness. Also, there is greater awareness of metabolic heterogeneity within tumors, with some cells using glycolysis as their main energy source, whereas others use oxidative phosphorylation. Recent studies on the tumor microenvironment have revealed the Reverse Warburg Effect in a subset of tumors, where metabolic coupling occurs between cancer-associated fibroblasts with high aerobic glycolysis, and tumor cells with increased mitochondrial oxidative phosphorylation. In this scenario, lactate (a high-energy metabolite) is transferred from fibroblasts to cancer cells, driving mitochondrial biogenesis in tumor cells. A similar physiological lactate-shuttle also exists in normal skeletal muscle and the brain.[2,3]

Arsenic Induces Mitochondrial Biogenesis, Leading to Carcinogenesis in Bowen's Disease

Arsenic induces skin cancer and also lung, liver, kidney, prostate, and bladder cancer.[4] Arsenic can dissociate from soil or rocks, and arsenic contamination of the aquifer water supply is a global public health crisis. The study by Lee et al[1] provides new important information on the mechanism(s) by which arsenic induces Bowen's disease, a type of skin carcinoma *in situ*.

The study by Lee et al[1] demonstrates that arsenic exposure is associated with increased mitochondrial biogenesis (Figure 1), as measured by mitochondrial DNA (mtDNA) copy number. In addition, there is increased expression of peroxisome proliferator-activated receptor gamma, co-activator 1 α (*PPARGC1A*), nuclear respiratory factor 1 (*NRF1*), and mitochondrial transcription factor A (*TFAM*; mtTFA protein), which are crucial genes for mitochondrial biogenesis, compared to normal healthy controls and patients with Bowen's disease without arsenic exposure. Keratinocytes exposed to low-dose arsenic have increased proliferation and expression of the previously stated mitochondrial biogenesis genes at the mRNA and protein levels. Low dose arsenic exposure leads to the up-regulation of mitochondrial oxidative phosphorylation enzyme subunits. Functionally, pharmacological down-regulation of mitochondrial function with oligomycin (a complex V inhibitor of electron transport chain involved in oxidative phosphorylation) abolishes the growth advantage provided by arsenic. Finally, this study shows that genetic down-regulation of mtTFA in keratinocytes leads to abrogation of the growth advantage provided by low dose arsenic.

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Address reprint requests to Ubaldo E. Martinez-Outschoorn, M.D., 925 Chestnut St. Suite 220A, Philadelphia, PA 19104; or Michael P. Lisanti, M.D., Ph.D., Kimmel Cancer Center, 223 S 10th St, BLSB Rms 909/939, Philadelphia, PA 19107. E-mail: ubaldo.martinez@gmail.com or michael.lisanti@kimmelcancercenter.org.

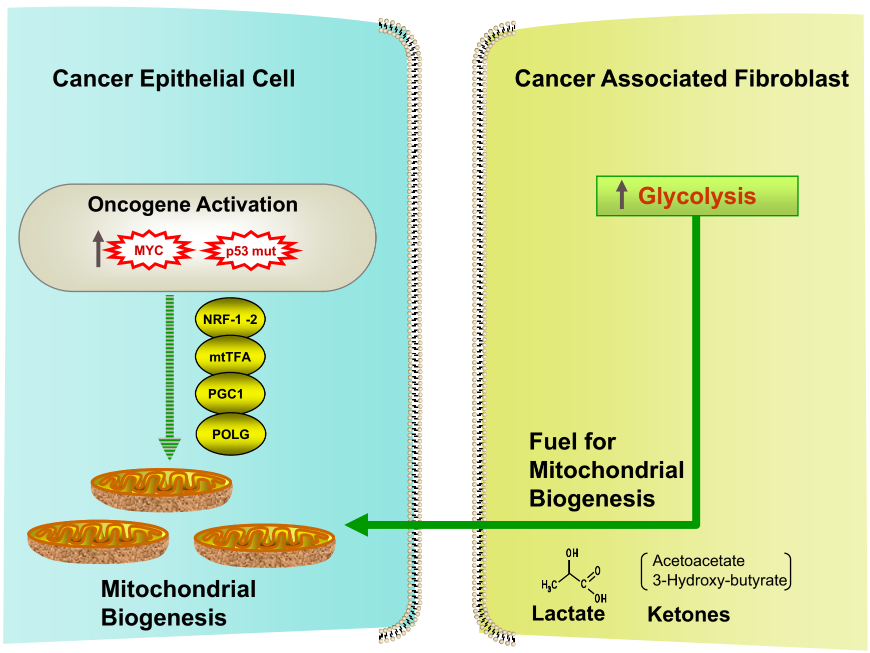

Figure 1. Mitochondrial biogenesis drives tumor cell proliferation. In this model, cancer-associated fibroblasts undergo aerobic glycolysis (the Warburg Effect), thereby providing recycled high-energy nutrients (such as L-lactate and ketone bodies) to fuel oxidative mitochondrial metabolism in adjacent cancer cells. Thus, in this paradigm, anabolic cancer cells undergo mitochondrial biogenesis and use oxidative mitochondrial metabolism to generate large amounts of ATP. This model is consistent with the current results of Lee et al[1] and has been referred to by others as The Reverse Warburg Effect.[24] Note also that overexpression of the MYC oncogene or inactivation of the p53 tumor suppressor drives mitochondrial biogenesis in cancer cells. Overall, this scheme provides an example of stromal-epithelial metabolic coupling in human tumors. More specifically, lactate (a high-energy metabolite) is transferred from fibroblasts to cancer cells, driving mitochondrial biogenesis in tumor cells. A similar physiological lactate-shuttle exists in normal skeletal muscle and the brain.[2,3]

In summary, the authors of this article have demonstrated that mitochondrial biogenesis is required for carcinogenesis induced by arsenic, and this has very important implications for public health, due to the widespread environmental exposure to this chemical element.

The Arsenic Paradox: Carcinogenesis Induced by Arsenic and Its Use as Anticancer Therapy

The study by Lee et al[1] adds to the growing body of literature studying the arsenic paradox. Arsenic is a carcinogen, but is also an effective cancer treatment. In this study, keratinocytes exposed to low levels of arsenic had increased proliferation, whereas this was abolished with high-dose arsenic. The arsenic paradox could be explained by the differing biological effects, depending on the concentration of arsenic. Arsenic impairs mitochondrial oxidative phosphorylation at high levels, but at low levels it promotes mitochondrial biogenesis.[1]

Inorganic arsenic is more toxic to cells than organic forms, and arsenic has two different oxidative states: As(III) and As(V). As(III) has more pro-apoptotic effects on cells. Arsenic's predominant intracellular effect is to bind to thiol or sulfhydryl groups within proteins. Arsenic inhibits antioxidant proteins, such as glutathione and thioredoxin, which leads to oxidative stress. The thioredoxin antioxidant system includes thioredoxin, thioredoxin reductase, and NADPH. Arsenic also up-regulates the pro-oxidant enzyme NADPH oxidase.[5] Arsenic generates increased reactive oxygen species, which at low and intermediate levels promotes mitochondrial biogenesis and is tumorigenic. However, at high levels it impairs mitochondrial function and is pro-apoptotic.

Arsenic in Cancer Therapy

Arsenic generates reactive oxygen species and, at high levels, leads to mitochondrial membrane potential destabilization, release of cytochrome *c* from mitochondria, with caspase activation, and ultimately cell death.[5,6] At high doses, aresenic down-regulates the anti-apoptotic proteins Bcl-2, Bcl-1, Bcl-xL, and Mcl-, and it activates the pro-apoptotic proteins Bax, Noxa, Bmf, and Bim.[7] Arsenic also activates JNK kinase and inhibits NF-κB–signaling by binding to the IKK activation loop, and promoting caspase-independent apoptosis.[8]

Although the majority of epidemiological data points to arsenic exposure as a cause of cancer,[4] a population cohort study from Denmark found that arsenic exposure may decrease skin cancer frequency, and there was no increase in malignancies.[9] It has been speculated that this may be due to differences in the level of exposure in that population compared to levels of exposure in Asia and Latin America, where a high incidence of arsenic-induced cancers has been noted.

As(III) in the form of arsenic trioxide is used as cancer chemotherapy. Arsenic trioxide is approved in the United States by the Food and Drug Administration for the treatment of acute promyelocytic leukemia. In addition to enhancing apoptosis, arsenic promotes the degradation by sumoylation of the pathogenic PML-RARA fusion protein in acute promyelocytic leukemia. Since arsenic is a known carcinogen, there are concerns about secondary malignancies after treatment with arsenic, but an increased incidence has not been shown. Arsenic trioxide is also being investigated in the treatment of solid tumors, but the appropriate subgroups of tumors remain to be defined since frequently very high doses of arsenic are required to induce apoptosis.

Mitochondrial Biogenesis as a Driver of Tumor Growth

The study by Lee et al.[1] shows that arsenic promotes tumor growth by up-regulating mitochondrial biogenesis and the implicated transcription factor mtTFA. The transcription of mtTFA is regulated by the transcription factors Nrf-1 and Nrf-2, which are also up-regulated by arsenic. The traditional view of cancer cell metabolism is that there is mitochondrial dysfunction. However, multiple studies have now shown that increased mitochondrial biogenesis promotes tumorigenesis, and loss of mtDNA leads to decreased tumorigenesis *in vivo* with impaired oxidative phosphorylation.[1011] Increased mtTFA is associated with cancer progression,[12,13] and loss of mtTFA inhibits K-Ras induced lung tumorigenesis.[14] Also, the mitochondrial protein p32 promotes oxidative phosphorylation and has been shown to be pro-tumorigenic *in vivo*.[15]

Low concentrations of arsenic induce MYC over-expression, and MYC expression leads to mitochondrial biogenesis.[16] Also, a decrease in p53 activity has been found in prostate cancers with chronic low-dose exposure to arsenic,[13] and mutations or knockdown of p53 promotes mitochondrial biogenesis.[17] In summary, MYC and p53 regulate mitochondrial biogenesis.[17]

Heterogeneity of Mitochondrial Metabolism in Cancers

There are two opposing views of cancer cell metabolism dating from the early days of this discipline. Otto Warburg hypothesized that irreversible respiration damage was the origin of cancer. He showed that cancer cells had impaired mitochondrial function with high glycolysis rates, high production of lactate, and impaired mitochondrial oxidative phosphorylation. Contrary to this view, Sidney Weinhouse showed that cancer cells could have normal oxidative phosphorylation if NAD+ was supplemented. Differences in cancer cell metabolism have been found repeatedly, especially when cells were studied *in vivo* as compared to *in vitro*.[18,19] For example, Warburg and colleagues studied Jensen sarcoma cells and concluded that there was high lactate production. It has later been shown that in Jensen sarcoma *in vivo*, net lactic acid production or utilization depends on the fed state of the animals and the concentrations of ketone bodies and lactate in the arterial circulation.[20] A review of the history of cancer metabolism has been recently published.[19] In summary, the majority of cancer cells studied in homotypic cell cultures have increased glycolysis, but there are numerous reports of cancer subtypes having increased mitochondrial oxidative phosphorylation.

More importantly, it is known that within tumors, there is heterogeneity of cell metabolism with metabolic coupling between well-oxygenated regions which have high rates of oxidative phosphorylation and regions which are hypoxic with high levels of glycolysis and lactate production. In fact, lactate is actively transported into oxygenated cells and serves as a substrate for mitochondrial oxidative phosphorylation.[21]

Also, the oncogenic role of the tumor microenvironment has gained widespread attention and the importance of stromal metabolism in tumorigenesis is becoming apparent. Caveolin-1 (Cav-1) is one of the important proteins that regulates stromal cancer metabolism. Cav-1 down-regulation in fibroblasts has been associated with impaired mitochondrial biogenesis in these cells. The lack of stromal Cav-1 is a strong prognostic and predictive biomarker in several human cancers, including breast and prostate cancers.[22,23]

It has been shown that tumors with loss of stromal Cav-1 have metabolic coupling between the stromal and the epithelial cell compartments,[24,25] with increased mitochondrial biogenesis in the epithelial cells and increased aerobic glycolysis, high lactate production, and mitochondrial dysfunction in the stromal cells (Figure 1). The mechanisms underlying this metabolic coupling include HIF1-α expression in the fibroblasts and TIGAR expression in the epithelial cancer cells. Studies examining HIF1-α over-expression in fibroblasts have shown that although this leads to aerobic glycolysis in the fibroblasts, it promotes cancer cell mitochondrial activity and tumor growth *in vivo*.[26] Lactate administration to cancer cell cultures also induces mitochondrial biogenesis, in a similar way to that induced by co-culture with fibroblasts.[25] Moreover, under defined experimental conditions, lactate administration inhibits the glycolytic enzymes hexokinase and phosphofructokinase.[27] Other types of stromal epithelial metabolic coupling have also been described with increased and decreased aerobic glycolysis in the cancer cells and fibroblasts, respectively.[28]

Interestingly, oncocytomas (benign tumors of the kidney, generally associated with an excellent overall prognosis) also show an increase in mitochondrial biogenesis. However, this appears to be a compensatory response to mutations in complex I of the mitochondrial respiratory chain (involved in electron transport).[29,30] As a consequence, oncocytomas contain defective mitochondria.[29,30] This may also explain why oncocytomas are benign, ie, because they cannot effectively use oxidative mitochondrial metabolism. This interpretation is consistent with the current results of Lee et al,[1] suggesting that proliferative or aggressive cancer cells benefit metabolically from functional mitochondria.

Summary

The article by Lee et al[1] sheds new light on how arsenic induces mitochondrial biogenesis in Bowen's disease. It is clear that not all cancer cells have the same metabolic profile and careful assessment of the metabolic state of a particular tumor is needed to understand the pathogenesis of different tumor subtypes, and for predicting clinical outcome, as well as treatment stratification. In accordance with this notion, lactate-induced mitochondrial biogenesis in cancer cells is associated with stemness, tumor recurrence, metastasis, and poor overall survival in breast cancer patients.[31]

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